

Bcl-2 AS A PROGNOSTIC FACTOR FOR SURVIVAL IN SMALL-CELL LUNG CANCER

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Abstract: *Background:* The expression of Bcl-2 oncoprotein is associated with inhibition of apoptosis and prolonged cell survival. The purpose of this study was to investigate Bcl-2 protein expression in patients with small-cell lung cancer (SCLC) in order to see if it was related to clinicopathological features and to prognosis.

Materials and methods: Forty patients with SCLC were stained immunohistochemically using specific monoclonal antibody (DAKO-Bcl-2, 124). Bcl-2 positivity was determined as detection of the oncoprotein in greater than 10% of neoplastic cells.

Results: Immunopositivity was present in 26 (60%) of SCLC patients. Twenty-three of 40 (57.5%) patients had limited disease at presentation, and 17 of 40 (42.5%) had extensive disease. There was not any correlation with Bcl-2 protein expression and clinicopathological parameters such as sex, age, smoking history and performance status. According to the extent of the disease, Bcl-2 expression was significantly higher in patients with extensive disease ($p < 0.009$). Bcl-2 expression was associated with significant shorter survival in patients with SCLC (Log Rank = -5.26 ; $p = 0.00001$). Cox regression analysis controlling for age, sex and tumor stage, confirmed that Bcl-2 ex-

pression (HR = 0.049 $p < 0.0001$) and N stage (HR = 0.152 $p < 0.012$) were an independent prognostic markers for poor prognosis

In *conclusion* Bcl-2 oncoprotein was expressed in most cases of SCLC and its expression may have prognostic importance.

Key words: Bcl-2 oncoprotein, small-cell lung carcinoma, immunohistochemistry, prognostic value.

Introduction

Lung cancer is divided into two broad categories: small cell lung cancer and non-small cell lung cancer, which have significant differences in clinical and biological behavior. Small cell lung carcinoma (SCLC), which comprises 20% of lung cancer, is characterized by a relentlessly progressive clinical course and by the early development of metastatic disease [1]. While SCLC is considered highly responsive to chemotherapy and radiotherapy, relaps despite treatment commonly occurs within 2 years. The 2 year survival in limited stage small cell lung cancer is only 20–30% [2], while the median survival in extensive stage small cell lung cancer is less than a year [3]. Given this dismal survival rate of the extended stage of the disease, other factors including clinical features at initial presentation, need to be evaluated in an attempt to define the outcome. Cytogenetic and molecular genetic analyses of SCLC have revealed that SCLC cells carry a number of chromosomal abnormalities [4].

Bcl-2 overexpression is common event in SCLC. The Bcl-2 proto-oncogene is involved in the 14; 18 translocation, a chromosomal abnormality present in 70% of follicular lymphomas and 20% of diffuse B-cell lymphomas [5]. The result of this translocation juxtaposes the Bcl-2 gene on chromosome 18 with the immunoglobulin heavy-chain (IGH) gene of chromosome 14. In consequences, as a result of the marked deregulation of the Bcl-2- IGH fusion gene, abnormally high levels of Bcl-2 protein are produced [6]. This protein protects cells from programmed cell death (apoptosis), and prolong cell survival by arresting cells in the G₀/G₁ phase of the cell cycle [7]. Korsmeyer has proposed that Bcl-2 is a member of a new category of oncogenes that is not involved in influencing cell proliferation but is involved in regulating cell death [8]. It is the only gene belonging to this category that has been identified. Although the expression of the Bcl-2 protein has been reported for a variety of human malignant tumors, including the lung, the precise biological role of Bcl-2 in the development of malignant tumors and predicting the survival is still controversial. In patients with lung cancer (LC) the most reports found that Bcl-2 expression was associated with favorable clinicopathological characteristics and prognosis [9, 10, 11, 12, 13], although the antiapoptotic action of Bcl-2 is expected to confer a sur-

vival advantage to the cancer cell. However, there have been some reports describing no significant correlation between Bcl-2 expression and prognosis [14, 15], or where Bcl-2 expression is linked with poor prognosis [16]. In this study we examined Bcl-2 protein expression in 40 patients with SCLC and assessed its correlation with clinocopathological features and its impact on survival of the patients.

Materials and methods

Study population

The study involved 40 consecutive patients with SCLC, who were diagnosed in the Institute for lung diseases and tuberculosis, Skopje, Macedonia, between March 2004 and January 2006. The diagnosis was established by histological examination of the tissue obtained during the fiber-optic bronchoscopy. All histological analyses and immunohistochemistry (ICH) were performed in the Institute for Pathology, Medical Faculty in Skopje, Macedonia. The staging procedure for the majority of patients was standardized including fiber-optic bronchoscopy, routine laboratory parameters, chest radiograph and CT, abdomen CT and bone scan.

Thirty-five patients (87.5%) were male and 5 patients were female (12.5%). According to the age 28 patients (70%) were younger than 65 years and 12 patients (30%) were older than 65. Smokers were 82.5%. The patients with SCLC are divided into the following two stages: limited disease (LD), which is disease confined to the ipsilateral hemithorax within a single radiotherapy port (corresponding to TNM stages I to IIIB), and extensive disease (ED), which includes evident metastases [17, 18]. Twenty three patients (57.5%) were with limited stage (LD), and seventeen (42.5%) were with extensive stage (ED) of the disease. All patients were treated and followed-up in the Institute for radiology and oncology, Medical Faculty, Skopje. The patients with limited disease were treated with combination of chemotherapy and radiotherapy, while the patients with extensive disease were treated with chemotherapy only.

The patients were followed up at least 24 months (from 1 to 48 months). The majority of patients were followed up regularly in a time frame of 2 to 3 months. The survival time was calculated from the date of histological diagnosis to the date of death or last follow-up.

Immunohistochemical Staining

The immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections using LCAB immunoperoxidase method with monoclonal antibody specific for Bcl-2 protein (clone Bcl-2-124; DAKO, Denmark).

Analyses was done with semiquantitative grading system. Bcl-2 expression was observed in the cytoplasm. Negative indicated no immunoreaction or < 10% of the tumor cells stained; (+) 10% to 25% staining; (++) 25% to 50% staining, and (+++): over 50% staining.

All of the tests were performed on biopsy specimens obtained before administration of any therapy.

Statistical Analysis

Statistical significance of the relationships between clinicopathological data and IHC were assessed by Mann-Whitney U test. The proportional hazard model was applied to examine whether Bcl-2 expression was an independent prognostic factor. The overall survival rate was calculated using the Kaplan-Meier method. The difference was considered to be statistically significant at $p < 0.05$.

Results

Twenty six patients (65%) of 40 patients with SCLC, showed positive cytoplasmic staining for Bcl-2. The results of Bcl-2 immunostaining and its relationship with clinicopathological features are summarized in Table 1.

Table 1 – Табела 1

Relationship between clinicopathological features and the expression of Bcl-2 in 40 patients with SCLC

Однос меѓу клиничкопатолошките карактеристики и експресијата на Bcl-2 при истражувањето кај 40 пациенти со СКБК

Features (n)	Bcl-2 (+) n (%)	Bcl-2 (-) n (%)	p Value
Total (40)	26 (65.0%)	14 (35%)	
Sex			
Male (35)	24 (92.3%)	11 (78.6%)	
Female (5)	2 (7.7%)	3 (21.4%)	NS
Age			
≤ 65 (28)	19 (73.1%)	9 (64.3%)	
> 65 (12)	7 (26.9%)	5 (35.7%)	NS
Smoking history			
Smoker (33)	21 (80.5%)	12 (85.7%)	
Nonsmoker (7)	5 (19.5%)	2 (14.3%)	NS

Performans status			
WHO 0 (11)	5 (19.2%)	6 (42.9%)	
WHO 1 (23)	15 (57.7%)	8 (57.1%)	
WHO 2 (3)	3 (11.5%)	/ (0%)	
WHO 3 (2)	2 (7.7%)	/ (0%)	
WHO 4 (1)	1 (26.9%)	/ (0%)	NS
Extent of the diseases			
Limited (23)	11 (42.3%)	12 (85.7%)	
Extensive (17)	15 (57.7%)	2 (14.3%)	P < 0.009
Stage			
IB (2)	/	2 (14.3%)	
IIA (1)	/	1 (7.1%)	
IIB (11)	6 (23.1%)	5 (35.7%)	
IIIA (9)	5 (19.2%)	4 (28.6%)	
IV (17)	15 (57.7%)	2 (14.3%)	P < 0.0004
T stage			
1 (1)	/ (0%)	1 (7.1%)	
2 (31)	20 (76.9%)	11 (78.7%)	
3 (6)	5 (19.2%)	1 (7.1%)	
4 (2)	1 (3.9%)	1 (7.1%)	NS
N stage			
0 (2)	/ (0%)	2 (14.3%)	
1 (15)	8 (30.8%)	7 (50.0%)	
2 (15)	10 (38.4%)	5 (35.7%)	
3 (8)	8 (30.8%)	/ (0%)	P < 0.001
M stage			
0 (24)	11 (42.3%)	13 (92.9%)	
1 (16)	15 (57.7%)	1 (7.1%)	P < 0.008

Note: statistical analysis was performed using Mann-Whitney U test

We didn't find any correlation with Bcl-2 protein expression and clinicopathological parameters such as sex, age, smoking history and performance status (classified according to WHO 0-IV). The most patients with extensive disease (15/17) showed Bcl-2 positive expression ($p < 0.009$). In the patients with limited disease, there was not differentiation between Bcl-2 positive and Bcl-2 negative expression. Analysis of TNM stage of the disease showed that 57.7% of patients with positive Bcl-2 status had advanced stage ($p < 0.0004$). When we analysed separately the correlation of Bcl-2 protein expression with T, N, M stages, we didn't find correlation with T stage; there was statistically

significant correlation of Bcl-2 expression with N and M stages: N0 stage was associated with negative Bcl-2 status ($p < 0.001$) opposite the data that Bcl-2 positive status was associated with present metastasis ($p < 0.008$).

In addition, survival time was significantly lower in patients whose tumors expressed Bcl-2 protein (Log Rank = -5.26 ; $p = 0.00001$): all patients with Bcl-2 positive expression died before 24 months (Figure 1). The median survival of patients with positive Bcl-2 status was 8 months, opposite 21 months for patients with negative status.

To determine which of the factors were independent prognostic factors of lung cancer-related death, a multivariate analysis using the Cox regression analysis was performed (Table 2). Factors in the analysis included age, sex, T, N, M stage and Bcl-2 expression. Examining the entire cohort, Bcl-2 status had the greatest impact on survival (HR = 0.04, CI = 0.0–0.2, $p = 0.000$), following by lymphonode involvement (HR = 0.15, CI = 0.0–0.6, $p = 0.01$). As a result, Bcl-2 status emerged as independent prognostic factor that might predict worse prognosis.

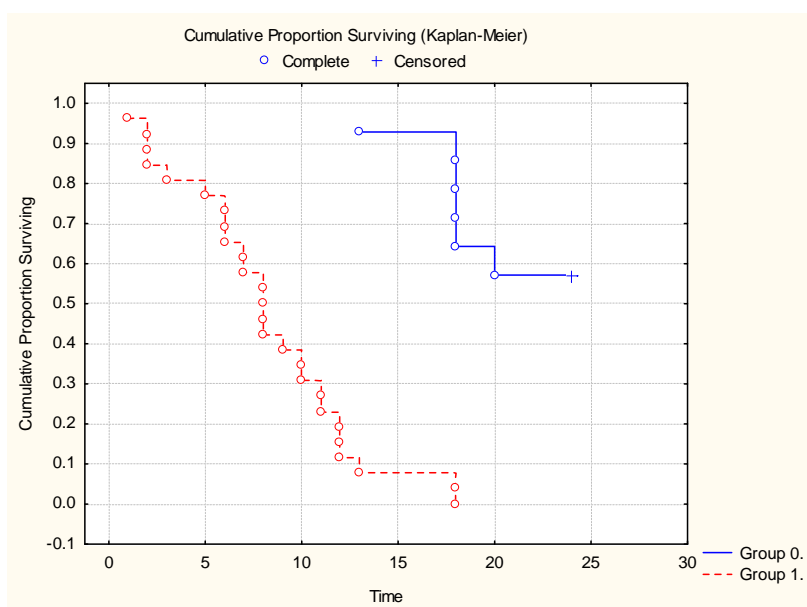


Figure 1 – Survival curves of patients with SCLC with Bcl-2 protein expression (dashed line) and without (solid line)

Слика 1 – Крива на преживување кај пациенти со СКБК со позитивна Bcl-2 протеинска експресија (испрекината линија) и без (целна линија)

Table 2 – Табела 2

Multivariate analysis of individual marker in 40 patients with SCLC
Мултиваријантна анализа на индивидуални маркери
кај 40 пациенти со СКБК

Marker	Hazard ratio	95% Confidence interval	p value
Sex	0.696	0.3–3.6	0.69
Age	0.993	0.9– 1.0	0.81
T stage			
2	0.77	0.1 – 4.4	0.98
3	0.36	0.0 – 2.6	0.31
N stage			
2	0.16	0.0 – 1.3	0.08
3	0.15	0.0 – 0.6	0.01*
M stage	4.16	0.0 – 4.4	0.33
Bcl-2	0.04	0.0 – 0.2	0.00*

Discussion

SCLC is an aggressive and highly metastatic diseases. Although SCLC tumors are initially responsive to chemotherapy, multi-drug resistance tends to develop after relapse and the majority of SCLC patients die of their disease within 2 years. One of the factors implicated in the resistance of SCLC to chemotherapy is the overexpression of the antiapoptotic protein Bcl-2 [19, 20]. Unlike other oncogenes, Bcl-2 promotes tumorigenesis by attenuating cell death as opposed to promoting cell proliferation [21].

The overexpression of Bcl-2 protein has been observed in a variety of cancers, including lymphoma, prostate, colon, and lung cancers, as well as many other types of solid tumor; it is associated with neuroendocrine differentiation and contributes to chemotherapeutic resistance [5, 22, 23, 24, 25, 26]. In SCLC patients, the overexpression of Bcl-2 has been reported to occur in 55% to 90% of all cases, and it has been suggested to be a key factor involved in both the genesis and maintenance of SCLC [4, 19, 20, 27, 28, 29, 30]. Our results which are in accordance with these from the literature, showed Bcl-2 positive expression in 65% of patients with SCLC.

Bcl-2 protein is more widely investigated in patients with non-small cell lung carcinoma (NSCLC). According to the results of meta analyses based on systematic review of the literature, overall expression of Bcl-2 protein in

NSCLC is 35%; in this analysis 32% of the squamous cell cancer and 61% of the adeno carcinoma expressed Bcl-2 [31].

Although there are a large number of studies of Bcl-2 expression, its value in predicting the survival of patients with lung cancer remains controversial. High level of Bcl-2 expression prevent cell death from a wide variety of cell stresses and cytotoxic chemicals including heat shock, ionizing radiation, excess calcium influx and a range of chemotherapeutic drugs. Therefore, tumors with high levels of Bcl-2 expression, especially in lymphoma, leukemia, and prostate cancer are associated with poor responses to chemotherapy and shorter disease-free and overall survival [32, 33, 34]. In contrast, though high level of Bcl-2 expression is correlated with poor clinical outcome, it has been associated paradoxically with a favorable response in lung, thyroid and breast cancer [10, 12, 35, 36, 37, 38].

The mechanism underlying the effect of Bcl-2 oncoprotein expression on tumor progression and prognosis remains essentially uncertain. Originally, the Bcl-2 gene product was implicated in oncogenesis because of its ability to prolong cell survival through the inhibition of apoptosis [21, 39]. The process of apoptosis involves many proteins (Bcl-2 family members) such as the anti-apoptotic proteins (Bcl-2, Bcl-X, Bfl-1) and the proapoptotic (Bax, Bak, Bad); [40]. These proteins can interact to regulate cellular apoptosis by balancing pro- and antiapoptotic mechanism. One distinguishing feature is that they share up to four Bcl-2 homology (BH) domains. The multidomains antiapoptotic Bcl-2 members inhibit cytochrome c release by blocking the activation of the multidomain proapoptotic proteins Bax and Bak. Bax and Bak are direct mediators of apoptosis and are absolutely required for the initiation of the mitochondrial apoptosis pathway [41, 42]. Thus, analyzing only one apoptotic protein can produce an incomplete appraisal of apoptosis and it would be interesting to conduct a survival analysis of a combination of these proteins.

Recent evidence suggests that cleavage of Bcl-2 by caspase-3 (mutations in domains) may either activate or inactivate essential function of Bcl-2. Cleavage of Bcl-2 by caspases has been shown to result in loss of the BH4 homology domain that is required for its antiapoptotic activity. Furthermore, the deletion of this domain has been shown to release a potent proapoptotic fragment. Thus, it is possible that Bcl-2 had lost its ability to suppress apoptosis due to the cleavage of caspase-3 [43]. Or, in those lung cancer cases where Bcl-2 expression is associated with favourable prognosis, it can be assumed that there are also Bcl-2 independent pathways that are involved in the regulation of apoptosis.

In the recent years, most of the preclinical studies have been focused in investigation of bioavailable inhibitors of Bcl-2 family proteins, which are associated with significant induction of apoptosis. These investigations should help in development of new anticancer strategies which will be of benefit to patients with SCLC [44, 45, 46].

In our study, Bcl-2 expression was associated with extensive stage of SCLC patients and with poor prognosis. The patients with positive Bcl-2 expression in tumors cells showed statistically lower survival than patients with negative Bcl-2 expression. By multivariate analysis controlling for age, sex, and tumor stage only Bcl-2 expression and lymphonode involvment were an independent markers of poor prognosis.

Conclusion

Disregulation of the genetic mechanisam controlling apoptosis is a critical step in the progression of SCLC, and Bcl-2 expression is involved in the pathogenesis of SCLC. We found that Bcl-2 expression in SCLC is independent poor prognostic marker and may help to identify patients who need more aggressive therapy.

REFERENCES

1. Elias A D. (1997): Small Cell Lung Cancer. *Chest*; 112: 2518–88.
2. Chute J.P., Venzon D.J., Hankins L., Okunieff P., Frame JN., Ihde DC., Johnson BE. (1997): Outcome of patients with small-cell lung cancer during 20 years of clinical research at the US National Cancer Institute. *Mayo Clin Proc*; 72: 901–12.
3. Seifter E.J., Ihde D.C. (1988): Therapy of small cell lung cancer: a perspective on two decades of clinical research. *Semin Oncol*; 15: 278–99.
4. Ikegaki N., Katsumata M., Minna J., Tsujimoto Y. (1994): Expression of Bcl-2 in small cell carcinoma cells 1. *Cancer Research*; 54: 6–8.
5. Pezzela F., Ralfkiaer E., Gatter K.C., Mason D.Y. (1990): The 14; 18 translocation in European cases of follicular lymphoma: comparison of Southern blotting and the polymerase chain reaction. *Br J Haematol*; 76: 58–64.
6. Tsujimoto Y., Finger L.A., Yunis J., Nowel P.C., Groce C.M. (1984): Cloning of the chromosome breakpoint of neoplastic B cells with the t(14; 18) chromosome translocation. *Science*; 226: 1097–9.
7. Cleary M.L., Smith S.D., Sklar J. (1986): Cloning and structural analysis of cDNAs for Bcl-2 and a hbrid Bcl-2 immunoglobulin transcript resulting from the t(14; 18) translocation. *Cell*; 47: 19–28.
8. Korsmeyer SJ. (1992): Bcl-2 initiates a new category of oncogenes: regulators of cell death. *Blood*; 80: 879–86.
9. Carvalho O.P.E., Ongelo A.L., Fabiola del Carlo B., Eduardo L., Leao L.E., Rodrigues O.L., Capelozzi VL. (2000): Useful prognostic panel markers to express the biological tumor status in resected lung adenocarcinoma. *Japanese Journal of Clin Oncology*; 30: 478–86.
10. Pezzella F., Turley H., Kuzu I., Tungekar M.F., Dnnill M.S., Pierce C.B., Harris A., Gatter K.C. (1993): Bcl-2 protein in non-small cell lung carcinoma. *N Engl J Med*; 329: 690–4.

11. Sloman A.D., Amico F., Yousem S.A. (1996): Immunohistochemical markers of prolonged survival in small cell carcinoma of the lung. An immunohistochemical study. *Arch Pathol Lab Med*; 120: 465–72.
12. Fontanini G., Vignati S., Bigini D., Mussi A., Lucchi M., Angeletti CA., Basolo F., Bevilacqua G. (1995): Bcl-2 protein: a prognostic factor inversely correlated to p53 in non-small-cell lung cancer. *Br J Cancer*; 71: 1003–7.
13. Ohsaki Y., Toyoshima E., Fujiuchi S., Matsui H., Hirata S., Miyokawa N., Kubo Y., Kikuchi K. (1996): Bcl-2 and p53 protein expression in non-small cell lung cancers: correlation with survival time. *Clin. Cancer Res*; 2: 915–20.
14. Anton R.C., Brown R. W., Younes M., Gondo M. M., Stephenson M. A., Cagle P.T. (1997): Absence of prognostic significance of Bcl-2 immunopositivity in non-small cell lung cancer: analysis of 427 cases. *Hum. Pathol*; 28: 1079–82.
15. Dosaka-Akita H., Katabami M., Hommura H., Fujioka Y., Katoh H., Kawakami Y. (1999): Bcl-2 expression in non-small lung cancers: higher frequency of expression in squamous cell carcinomas with earlier pT status. *Oncology*; 56: 259–64.
16. Kim Y.C., Park K.O., Kern J.A., Park C.S., Lim S.C., Jang A.S., Yang J.B. (1998): The interactive effect of Ras, HER2, P53 and Bcl-2 expression in predicting the survival of non-small cell lung cancer patients. *Lung Cancer*; 22: 181–90.
17. Anthony D., Elias M.D. (1997): Small Cell Lung Cancer. State-of-the-Art Therapy in 1996. *Chest*; 112: 251S–8S.
18. Kurup A., Hanna H.N. (2004): Treatment of small cell lung cancer. *Critical Reviews in Oncology/Hematology*; 52: 117–126.
19. Takayama K., Ogata K., Nakanishi Y., Yatsunami J., Kawasaki M., Hara N. (1996): Bcl-2 expression as a predictor chemosensitivities and survival in small cell lung cancer. *Cancer J. Sci Am*; 2: 212–6.
20. Higashiyama M., Doi O., Kodama K., Yokouchi H., Tateishi R. (1995): High prevalence of Bcl-2 oncoprotein expression in small cell lung cancer. *Anticancer Res*; 15: 503–5.
21. Reed JC. (1995): Bcl-2: prevention of apoptosis as a mechanism of drug resistance. *Hemat Oncol Clin North Am*; 9: 451–73.
22. Reed JC. (1997): Bcl-2 family proteins: Strategies for overcoming chemoresistance in Cancer. Apoptosis: pharmacological implications and therapeutic opportunities. *Advances In Pharmacology, New York: Academic Press*; 41: 501–6.
23. Leek R.D., Kaklamamis L., Pezzella Gatter K.C., Harris A.L. (1994): Bcl-2 in normal human breast and carcinoma, associated with estrogen receptor positive, epidermal growth factor receptor –negative tumor and in situ cancer. *Br J Cancer*; 69: 135–9.
24. Oefner D., Riehemann K., Maier H., Riedmann B., Nehoda H., Totsch M. *et al.* (1995): Immunohistochemically detectable Bcl-2 expression in colorectal carcinoma: correlation with tumor stage and patient survival. *Br J Cancer*; 72: 981–5.
25. Pezzella F., Turley H., Kuzu I., Tungekar M.F., Dnnill M.S., Pierce C.B. *et al.* (1993): Bcl-2 protein in non-small cell lung carcinoma. *N Engl J Med*; 329: 690–4.
26. Krug M.L., Miller V., Filippa A.D., Venkatraman E., Kris G.M. (2003): Bcl-2 and bax expression in advanced non-small cell lung cancer: lack of correlation

with chemotherapy response or survival in patients treated with docetaxel plus vinorelbine. *Lung cancer*; 39: 139–43.

27. Ben-Ezra J.M., Kornstein M.J., Grimes M.M., Krystal G. (1994): Small cell carcinomas of the lung express the Bcl-2 protein. *Am J Pathol*; 145: 1036–40.

28. Yan J.J., Chen F.F., Tsai Y.C., Jin Y.T. (1996): Immunohistochemical detection of Bcl-2 protein in small cell lung carcinomas. *Oncology*; 53: 6–11.

29. Paik H.K., Park H.Y., Ryoo Y.B., Yang H.S., Lee C.J., Kim H.C. *et al.* (2006): Prognostic Value of Immunohistochemical Staining of p53, Bcl-2, and Ki-67 in Small Cell Lung Cancer. *J Korean Med Sci*; 21: 35–9.

30. Zareu M., Vinholes J.J., Zettler C.G. (2003): P53 and Bcl-2 protein expression and its relationship with prognosis in small-cell lung cancer. *Clin Lung Cancer*; 4: 298–302.

31. Martin B., Paesmans M., Berghmans T., Branle F., Ghisdal L., Mascaux C. *et al.* (2003): Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *British Journal of Cancer*; 89: 55–64.

32. Charalambous G.K., Gomatos I.P., Konstadoulakis M.M., Messaris E.G., Manouras A.J., Apostolou A.E. *et al.* (2000): Protein expression of bax, Bcl-2 and p53 in patients with Non-Hodgkin's Gastric Lymphoma: prognostic significance. *World J. Surg*; 24: 608–14.

33. Campos L., Roualut J-P., Sabido O. (1993): High expression of Bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood*; 81: 3091–6.

34. Yaren A., Oztop I., Kargi A., Ulucus C., Onen A., Sanli A. (2006): Bax, Bcl-2 and c-kit expression in non-small-cell lung cancer and their effects on prognosis. *International Journal of Vlinical Practice*; 60: 675–82.

35. Higashiyama M., Doi O., Kodama K., Yokouchi H., Nakamori S., Tateishi R. (1997): Bcl-2 oncoprotein in surgically resected non-small cell lung cancer: possibly favourable prognostic factor in association with low incidence of distant metastasis. *J. Surg. Oncol.*; 64: 48–54.

36. Ritter J.H., Dresler C.M., Wick M.R. (1995): Expression of Bcl-2 protein in stage T1N0M0 non-small cell lung carcinoma. *Hum. Pathol.*; 26: 1227–32.

37. Higashiyama M., Doi O., Kodama K., Yokouchi H., Nakamori S., Tateishi R. (1998): Bcl-2 oncoprotein in surgically resected nonsmall cell lung cancer: Possibly favorable prognostic factor in association with low incidence of distant metastasis. *Surgical Oncology*; 64: 48–54.

38. Leek R.D., Kaklamamis L., Pezzella Gatter K.C., Harris A.L. (1994): Bcl-2 in normal human breast and carcinoma, associated with estrogen receptor positive, epidermal growth factor receptor –negative tumor and in situ cancer. *Br J Cancer*; 69: 135–9.

39. Adams J.M., Cory S. (1998): The Bcl-2 protein family: arbiters of cell survival. *Science*; 281: 1322–6.

40. Daniel C.J., Smythe W.R. (2004): The role of Bcl-2 family members in non-small cell lung cancer. *Thoracic and Cardiovascular Surgery*; 16: 19–27.

41. Cory S., Adam J.M. (2002): The Bcl-2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*; 2 (9): 647–56.
42. Green D.R., Reed J.C. (1998): Mitochondria and Apoptosis. *Science*; 281: 1309–12.
43. Cheng H.Y., Kirsch D.G., Clem R.J., Ravi R., Kastan M.B., Bedi A. *et al.* (1997): Conversion of Bcl-2 to a Bax-like Death Effector by Caspase. *Science*; 278: 1966–8.
44. Mortenson M.M., Schlieman G.M., Virudachalm S., Lara N.P., Gandara G.D., Davies M.A. *et al.* (2005): Reduction in Bcl-2 leveles by 26S proteasome inhibition with bortezomib is associated with induction of apoptosis in small cell lung cancer. *Lung Cancer*; 49: 163–170.
45. Rudin M.C., Kozloff M., Hoffman C.P., Edelman J.M., Karnauskas R., Tomek R. *et al.* (2004): Phase I Study of G3139, a Bcl-2 Antisense Oligonucleotide, Combined With Carboplatin and Etoposide in Patients With Small-Cell Ling Cancer. *Journal of Clinical Oncology*; 22: 1110–1117.
46. Tahir K.S., Yang X., Anderson G.M., Morgan-Lape E.S., Sarthy V.A., Chen J. *et al.* (2007): Influence of Bcl-2 Family Members on the Cellular Response of Small-Cell Ling Cancer Cell Lines to ABT-737. *Cancer Research*; 67: 1176–1183.

Резиме

Bcl-2 КАКО ПРОГНОСТИЧКИ ФАКТОР НА ПРЕЖИВУВАЊЕ КАЈ СИТНО-ЌЕЛИСКИ БЕЛОДРОБЕН КАРЦИНОМ

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Цели: Експресијата на Bcl-2 онкопротеинот е поврзана со инхибиција на апоптозата и со пролонгирано ќелиско преживување. Целта на оваа студија е да се одреди Bcl-2 протеинската експресија кај пациенти со ситно-ќелиски белодробен карцином (СКБК) и да се одреди дали е во корелација со клиничко-патолошките карактеристики и со прогнозата на болеста.

Материјал и методи: Кај 40 пациенти со СКБК беа направени имунохистохемски анализи со примена на специфични моноклонални антители (ДАКО-Vcl-2, 124). Vcl-2 позитивен наод е дефиниран како присуство на онкопротеинот во повеќе од 10% од малигните ќелии.

Резултати: Имунопозитивен наод е добиен кај 26 (60%) пациенти со СКБК. Ограничена болест имаа 23 од 40 пациенти (57,5%), додека 17 (42,5%) беа со проширена форма на болест. Не постоеше корелација помеѓу Vcl-2 протеинската експресија и клиничко-патолошките параметри како пол, возраст, пушење, статусот на пациентот и туморската диференцијација. Според проширеноста на болеста, Vcl-2 експресијата беше значително повисока кај пациентите со проширена форма на болест ($p < 0,009$). Vcl-2 експресијата беше поврзана со значително пократко преживување кај пациентите со СКБК (Log Rank = -5.26 ; $p = 0,00001$). Мултиваријантната анализа која ги опфаќаше полот, возраста и стадиумот на болеста покажа дека експресијата на Vcl-2 онкопротеинот (HR = $0,049$ $p < 0,0001$) и N стадиумот (HR = $0,152$ $p < 0,012$) се независни прогностички маркери кај пациентите со СКБК.

Заклучок: Како заклучок, Vcl-2 онкопротеинот е присутен кај поголем број случаи со СКБК и има значителна прогностичка вредност.

Клучни зборови: Vcl-2 онкопротеин, ситно-ќелиски белодробен карцином, имунохистохемски анализи, прогностичка вредност.

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